

Hand-pairing of *Papilio glaucus glaucus* and *Papilio pilumnus* (Papilionidae) and hybrid survival on various food plants

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Abstract. Hand-pairing of a female *Papilio glaucus* with a male *P. pilumnus* resulted in the hatching of 69 larvae. Hybrid larvae survived on species of Lauraceae, and also on species of Rutaceae and Magnoliaceae. Hybrid larvae did not initiate feeding on black cherry. Both larvae and the adult males that were produced were intermediate between the two species in a variety of morphological traits.

Introduction

The three-tailed swallowtail, *Papilio pilumnus* Boisduval occurs from southern Arizona and Texas southward to Guatemala (Beutelspacher and Howe, 1984). Howe (1975) observed oviposition on a species of *Litsea* (Lauraceae) in Chiapas, Mexico, however relatively little is known about its larval stages or field biology (but see Scott, 1986). Because of its superficial resemblance to adult tiger swallowtails (Fig. 1). *P. pilumnus* generally had been considered to be a member of the *Papilio glaucus* L. species group until Brower (1959) placed it with the *Papilio troilus* L. group. Three factors support the placement of *P. pilumnus* with the *P. troilus* group. The male genitalia are more like those of *P. troilus* (Brower, 1959). As originally described by Schaus (1884), *P. pilumnus* larvae more closely resemble *P. troilus* and *P. palamedes* than any of the *P. glaucus* group species. In addition, pupal color and morphology (Schaus, 1884) are more like *P. troilus* and *P. palamedes*. Tyler (1975), however, suggested that *P. pilumnus* is transitional between the *P. glaucus* and *P. troilus* species groups.

In our ongoing studies of the physiological and biochemical mechanisms of differential foodplant use in North American *Papilio glaucus* and *Papilio troilus* species and subspecies, we have hand-paired various taxa in more than 4000 crosses (see Scriber, 1987a,b,c). Among the most interesting were our pairings of virgin *Papilio glaucus* females with field captured *P. pilumnus* males. In this paper, we describe the hybrid offspring of these pairings and their abilities to use potential foodplants.

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Methods:

To obtain virgin females, we first collected adult females of *Papilio glaucus*, *P. troilus*, and *P. alexiaries* from the field. Females were allowed to oviposit individually on acceptable foodplant leaves (black cherry and sassafras) kept fresh by water-filled aquapics®. Females were housed in clear plastic boxes (12cm x 20cm x 30cm) heated by a 100w incandescent lightbulb placed approximately 0.5m from the boxes. Eggs were removed on leaves at 2 day intervals after oviposition and neonate larvae were subsequently reared on excised leaves of various species of plants (in 4cm x 15cm petri dishes with screened ventilation) to pupation. Larvae were reared in controlled environment growth chambers (at 16:8 photo/scotophase with a corresponding temperature regime of 23.5/19.5 degrees C). Foodplant leaves were kept turgid by use of water-filled aquapics (Scriber, 1977), and changed as needed. A mixture of healthy-looking mature (fully-expanded) leaves and younger leaves were used for neonate studies. After weighing, pupae were individually placed in 14 cm screen cages until adult emergence.

Hand-pairings of virgin female butterflies to field-collected males were conducted as in Clarke & Sheppard (1956) with the pair hanging in a screen cylinder (approximately 12cm tall by 14cm diameter) covered by the top and bottom of a petri dish. Females that had been in copulation at least 30 minutes were set up in the oviposition boxes as described above. Newly eclosed larvae were individually transferred with a camel hair brush and distributed among the various foodplants. After females died, they were dissected and examined for spermatophores.

Results:

Males and females of *Papilio pilumnus* were collected by M. Evans, D. Robacker, and W. Warfield in the states of Nuevo Leon and Tamaulipas in northeastern Mexico and brought to the laboratory. One *P. pilumnus* female produced 2 eggs, but no larvae. A second *P. pilumnus* female laid 5 eggs. The single larva did not survive on red bay. A third *P. pilumnus* female laid 4 eggs; the single hatchling developed on sassafras. Although a total of five hand-pairings lasted 30 minutes or longer, in three, no spermatophore was passed (one with *P. troilus*, #4245, one with *P. alexiaries*, #3301, and one with *P. glaucus*, #4235). Only one pairing with a *P. glaucus* (#4231) produced fertile eggs. Of a total of 108 eggs, 69 larvae eclosed, 7 died while crawling out of their eggs, 7 additional eggs appeared to be fertile but produced no larvae, and 25 eggs appeared to be infertile.

No neonate hybrid larvae survived on black cherry, paper birch, quaking aspen, or sycamore (Rosaceae, Betulaceae, Salicaceae, and Platanaceae, respectively; see Table 1). There was no indication that the larvae initiated feeding on these hosts. However, species from the Rutaceae (hop tree), Magnoliaceae (sweet bay and tulip tree), and Lauraceae (spicebush, red bay, and sassafras) were accepted by the neonate larvae and nearly 50% survived to the second instar (Table 1). Larvae surviving to the second instar on plants other than sassafras and

Table 1. Neonate larval survival of F₁ hybrid larvae of the cross (*Papilio glaucus* female) x (*P. pilumnus* male).

Plant Species	Plant Family	Surviving(n) to second instar	Total set up	% Survival
<i>Prunus serotina</i> Ehrh. (Black Cherry)	(Rosaceae)	0	27	0
<i>Betula papyrifera</i> Marsh. (Paper Birch)	(Betulaceae)	0	6	0
<i>Populus tremuloides</i> Michx. (Quaking Aspen)	(Salicaceae)	0	3	0
<i>Platanus occidentalis</i> L. (Sycamore)	(Platanaceae)	0	3	0
<i>Ptelea trifoliata</i> L. (Hop Tree)	(Rutaceae)	1	2	50
<i>Magnolia virginiana</i> L. (Sweetbay)	(Magnoliaceae)	1	4	25
<i>Liriodendron tulipifera</i> L. (Tulip Tree)	(Magnoliaceae)	4	8	50
<i>Lindera benzoin</i> (L.) Blume (Spicebush)	(Lauraceae)	1	4	25
<i>Persea borbonia</i> (L.) Spreng. (Red Bay)	(Lauraceae)	2	6	33
<i>Sassafras albidum</i> (Nutt.) Nees (Sassafras)	(Lauraceae)	5	6	83

tulip tree were switched to one of these species for rearing. Of the 14 larvae surviving the feeding trials, 5 successfully pupated (Table 2). All resulting adults were male. No attempt to determine the fertility of these males was made.

The adult male hybrids are intermediate in wing pattern and shape between the *P. glaucus glaucus* males and *P. pilumnus* males on both the dorsal (Fig. 1a, b, c) and ventral (Fig. 2a, b, c) sides. It is also clear that the final larval instar reflects a composite of traits from each species. Unlike *Papilio glaucus* which is a solid green color (Fig. 3a), the hybrid larva (Fig. 3b) has a yellow line running along the side of the body with a brown ventral color and a series of blue spots on the abdominal segments just below this line as described for *P. pilumnus* (Fig. 3c) (Schaus, 1884; Brower, 1959), *P. palamedes* (Fig. 3d), and *P.*

Table 2. A summary of data for five *P. glaucus* x *P. pilumnus* larvae which pupated.

Larval Hatch Data (Aug 1986)	Larval Food	Pupation Date (Sept.)	Larval Duration (days)	Pupal wt. (gm.)	Adult Emergence Date (1986) (All Males)	Pupal Duration (days)
18	Redbay-Tulip Tree	20	33	no. wt.	1 Oct.	11
18	Tulip Tree	10	23	0.3229	Dead as a pharate adult by late Oct	—
19	Spicebush-Sassafras	26	38	0.4692	7 Oct Deformed Adult	11
19	Sassafras	12	24	0.5089	23 Sept. Deformed Adult	11
19	Sassafras	12	24	0.5174	23 Sept. Deformed Adult	11

troilus (Fig. 3e). Unlike *P. palamedes*, *P. troilus*, and *P. pilumnus*, the hybrid larva has a false thoracic eyespot without a solid black center (Fig. 3b, 3c, 3d, 3e) which closely resembles the false eyespot of *P. glaucus* (Fig. 3a). The brown larval stage (before pupation) of the hybrid larva (Fig. 4) lacks the transverse yellow at the base of the thorax which characterizes *P. glaucus* (Fig. 3a). the hybrid pupae were small (presumably due to nutritional factors) and were more *troilus*-like than *glaucus*-like in general shape (Fig. 5). As described for *P. pilumnus* pupae (Brower, 1959), these hybrid pupae were pinkish in color and laterally ridged, which is unlike those of all *P. glaucus* group species.

Discussion:

The survival and developmental compatibility in these *P. glaucus*/*P. pilumnus* hybrid genomes were surprising, especially when compared with other interspecific pairings we have conducted between various *P. glaucus* and *P. troilus* species group members. Viability of the *glaucus*/*pilumnus* hybrids was comparable with that of interspecific hybrids

within the *glaucus* group but greater than that of previous *glaucus* group/*troilus* group hybrids. For example, the average viability (larvae/eggs) of the two *glaucus/pilumnus* pairings here was 35.2% (70.4, 0), compared to pairings of *P. glaucus* females with males of *P. multicaudatus* (n=10; x=44.0%) *P. eurymedon* (n=18; x=30.2%), *P. rutulus* (n=8; x=31.1%), and *P. alexiaries* (n=15; x=56.1%). Although attempted numerous times only one successful pairing of a *P. glaucus* female with a *P. troilus* male has ever been obtained (i.e. copulation for more than 30 minutes), and none of the eggs produced hatched. We have achieved 3 successful hand-pairings of *P. palamedes* males with female *P. glaucus*. One such pairing produced viable offspring. It should be pointed out, however, that a number of factors other than genetic compatibility are likely to be involved in determining egg viability, and considerable caution in the interpretation of fertility data is advised (eg. see Lederhouse and Scriber, 1987).

The ability of these hybrid larvae to eat, survive, and grow on the Lauraceae was not surprising since this is the only family the related *P. troilus* and *P. palamedes* may actually utilize (Scriber, 1986), and *Papilio glaucus* can utilize lauraceous species to a certain extent (with the exception of red bay) (Scriber, et al. 1975; Scriber, 1973, 1984, 1986, 1987c). The ability of hybrid larvae to use Rutaceae and Magnoliaceae may reflect the contribution of *P. g. glaucus* to their genome or latent ancestral capabilities. Although it generally has been presumed that the Lauraceae were ancestral foodplants with a key role in the evolution of *Papilio* (Forbes 1932, 1958; Munroe, 1948, 1960), the Rutaceae (Munroe and Ehrlich, 1960; Scriber, 1972; Hancock, 1983;) and the Magnoliaceae (Dethier, 1941, Ehrlich and Raven, 1965) could be equally important. Feeny, et. al. (1983) summarized the potential chemical common denominators among these plant families.

Perhaps the most interesting aspect of these hybrid *glaucus/pilumnus* foodplant bioassays were the plants that larvae did not successfully use (Table 1). In particular, black cherry (*Prunus serotina*) and paper birch (*Betula papyrifera*) are two of the plant species that all members of the *Papilio glaucus* species group readily consume (including *P. rutulus*, *P. eurymedon*, *P. glaucus glaucus*, *P. g. canadensis* and *P. alexiaries*; Scriber, 1987b, 1987c). The hybrid larvae did not initiate feeding on either black cherry or paper birch apparently not recognizing them as potential hosts. Unlike the hybrid transfer of detoxication abilities between Salicaceae feeders (*P. rutulus*, *P. eurymedon* and *P. g. canadensis*) and Magnoliaceae feeders (*P. g. glaucus*, *P. g. australis*, *P. alexiaries*; Scriber, 1987a), it remains unclear if the *P. glaucus* abilities to use Betulaceae and Rosaceae were transferred to the hybrid larvae (*glaucus* x *pilumnus*) in this study. Sample sizes are low, and additional crosses seem warranted because there is an interesting genetic story regarding detoxication (and perhaps phylogenetic history) to be worked out here.

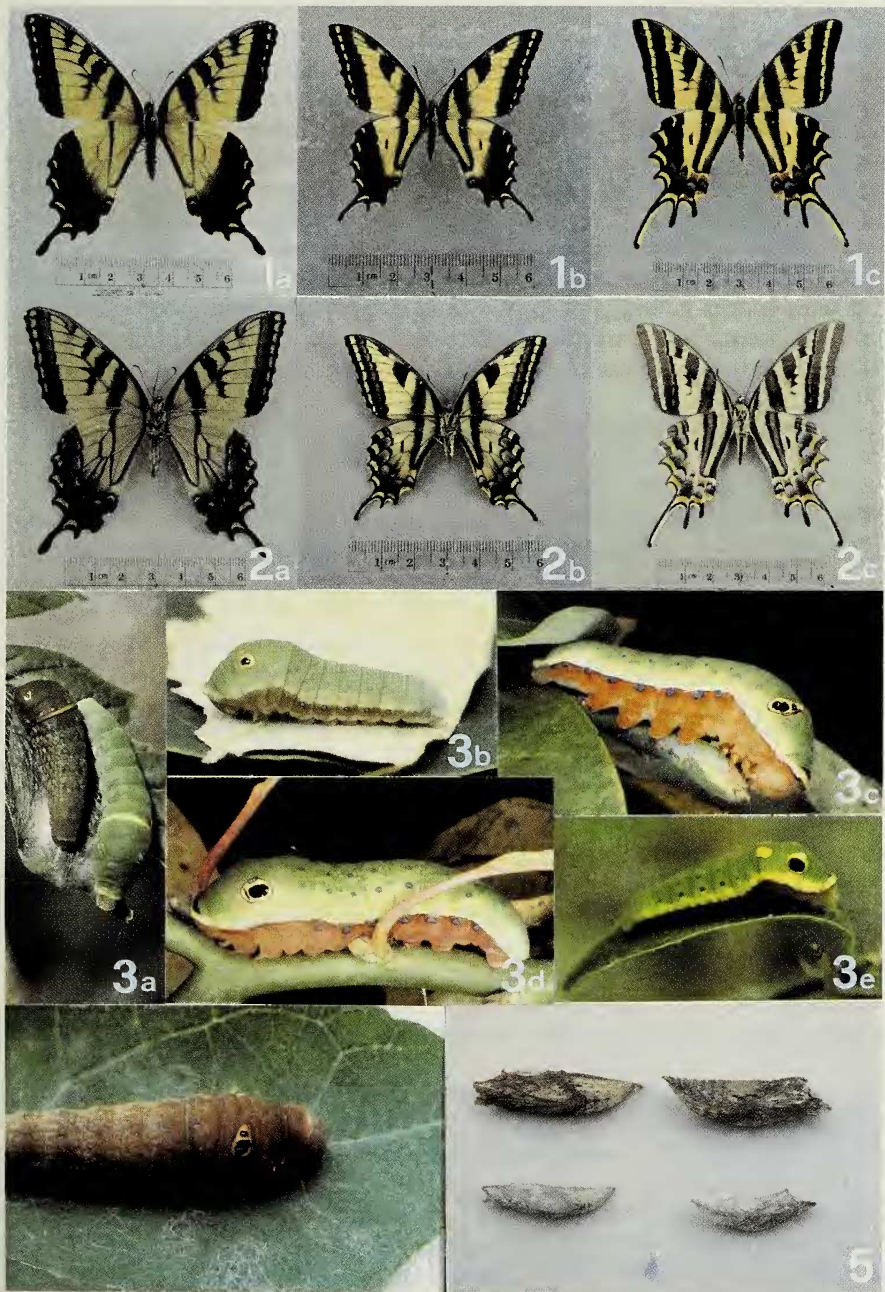


Fig. 1. Dorsal view of adult males of: a) *P. g. glaucus* b) hybrid *P. g. glaucus* (female) x *P. pilumnus* (male) c) *P. pilumnus*
 Fig. 2. Ventral view of specimens in Fig. 1
 Fig. 3. Final (5th) instar of: a) *P. g. glaucus*, brown and green morphs. b) hybrid *P. g. glaucus* x *P. pilumnus* c) *P. pilumnus* d) *P. palamedes* e) *P. troilus*
 Fig. 4. Hybrid larva (*P. g. glaucus* x *P. pilumnus*) that has turned brown prior to pupation
 Fig. 5. Ventral and lateral view of the hybrid pupae (bottom) contrasted to the same views of *P. glaucus* pupae (top)

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